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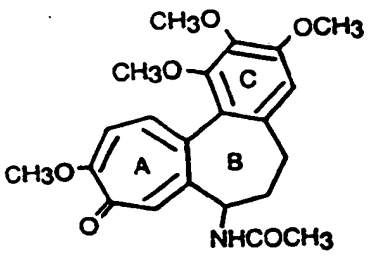

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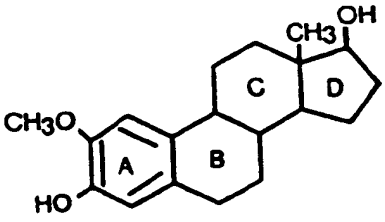
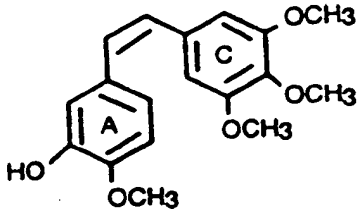


2-METHOXYESTRADIOL



COMBRETASTATIN A-4

(57) Abstract

The application discloses methods of making medicaments for treating mammalian diseases characterized by abnormal cell mitosis by administering estradiol derivatives including those comprising colchicine or combretastatin A-4 structural motifs of general formulae found above in a dosage sufficient to inhibit cell mitosis. The application discloses novel compounds used in the methods.

ESTROGENIC COMPOUNDS AS ANTI-MITOTIC AGENTS

Background of the Invention

This invention relates to treating disease states characterized by abnormal cell mitosis.

Cell mitosis is a multi-step process that includes cell division and replication (Alberts, B. et al. In *The Cell*, pp. 652-661 (1989); Stryer, E. *Biochemistry* (1988)). Mitosis is characterized by the intracellular movement and segregation of organelles, including mitotic spindles and chromosomes. Organelle movement and segregation are facilitated by the polymerization of the cell protein tubulin. Microtubules are formed from α and β tubulin polymerization and the hydrolysis of GTP. Microtubule formation is important for cell mitosis, cell locomotion, and the movement of highly specialized cell structures such as cilia and flagella.

Microtubules are extremely labile structures that are sensitive to a variety of chemically unrelated anti-mitotic drugs. For example, colchicine and nocadazole are anti-mitotic drugs that bind tubulin and inhibit tubulin polymerization (Stryer, E. *Biochemistry* (1988)). When used alone or in combination with other therapeutic drugs, colchicine may be used to treat cancer (WO-9303729-A, published March 4, 1993; J03240726-A, published October 28, 1991), alter neuromuscular function, change blood pressure, increase sensitivity to compounds affecting sympathetic neuron function, depress respiration, and relieve gout (*Physician's Desk Reference*, Vol. 47, p. 1487, (1993)).

Estradiol and estradiol metabolites such as 2-methoxyestradiol have been reported to inhibit cell division (Se gers, J.C. et al. *J. Steroid Biochem.* 32,

change (increase or decrease) in estrogen receptor binding, improved absorbtion, transport (e.g. through blood-brain barrier and cellular membranes), biological stability, or decreased toxicity. I have also discovered certain compounds useful in the method, as described by the general formulae of the claims.

A mammalian disease characterized by undesirable cell mitosis, as defined herein, includes but is not limited to excessive or abnormal stimulation of endothelial cells (e.g., atherosclerosis), solid tumors and tumor metastasis, benign tumors, for example, hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas, vascular malfunctions, abnormal wound healing, inflammatory and immune disorders, Bechet's disease, gout or gouty arthritis, abnormal angiogenesis accompanying: rheumatoid arthritis, psoriasis, diabetic retinopathy, and other ocular angiogenic diseases such as retinopathy of prematurity (retrolental fibroplasic), macular degeneration, corneal graft rejection, neovascular glaucoma and Osler Weber syndrome. Other undesired angiogenesis involves normal processes including ovulation and implantation of a blastula. Accordingly, the compositions described above can be used to block ovulation and implantation of a blastula or to block menstruation (induce amenorrhea).

The bond indicated by C•••C is absent or, in combination with the C---C bond is the unit HC=CH.

Other features and advantages of the invention will be apparent from the following description of preferred embodiments thereof.

Description of the Preferred Embodiments

The drawings are first described.

Fig. 1 is a graph illustrating th inhibition of tubulin polymerization by 2-methoxyestradiol described by Example 1 below.

Figure 3, part I, depicts the chemical formulae of colchicine, 2-methoxyestradiol and combretastatin A-4. Figure 3, part IIa-d, illustrates estradiol derivatives that comprise structural motifs found in colchicine or combretastatin A-4. For example, part II a-c shows estradiol derivatives with an A and/or B ring expanded from six to seven carbons as found in colchicine and part IId depicts an estradiol derivative with a partial B ring as found in combretastatin A-4. Each C ring of an estradiol derivative, including those shown in Figure 3, may be fully saturated as found in 2-methoxyestradiol. R_{1-6} represent a subset of the substitution groups found in the claims. Each $R_1 \rightarrow R_6$ can independently be defined as $-R_1$, OR_1, $-OCOR_1$, $-SR_1$, $-F$, $-NHR_2$, $-Br$, $-I$, or $-C\equiv CH$.

Anti-mitotic Activity *In Situ*

Anti-mitotic activity is evaluated *in situ* by testing the ability of an improved estradiol derivative to inhibit the proliferation of new blood vessel cells (angiogenesis). A suitable assay is the chick embryo chorioallantoic membrane (CAM) assay described by Crum et al. *Science* 230:1375 (1985). See also, U.S. Patent 5,001,116, hereby incorporated by reference, which describes the CAM assay. Briefly, fertilized chick embryos are removed from their shell on day 3 or 4, and a methylcellulose disc containing the drug is implanted on the chorioallantoic membrane. The embryos are examined 48 hours later and, if a clear avascular zone appears around the methylcellulose disc, the diameter of that zone is measured. Using this assay, a 100mg disk of the estradiol derivative 2-methoxyestradiol was found to inhibit cell mitosis and the growth of new blood vessels after 48 hours. This result indicates that the anti-mitotic action of 2-methoxyestradiol can inhibit cell mitosis and angiogenesis.

graft r jection, neuroscular glacoma and Oster Webber syndrome.

Improved Estradiol Derivative Synthesis

Known compounds that are used in accordance with the invention and precursors to novel compounds according to the invention can be purchased, e.g., from Sigma Chemical Co., St. Louis, Steroloids and Research Plus. Other compounds according to the invention can be synthesized according to known methods from publicly available precursors.

The chemical synthesis of estradiol has been described (Eder, V. et al., *Ber* 109, 2948 (1976); Oppolzer, D.A. and Roberts, D.A. *Helv. Chim. Acta.* 63, 1703, (1980)). Synthetic methods for making seven-membered rings in multi-cyclic compounds are known (Nakamuru, T. et al. *Chem. Pharm. Bull.* 10, 281 (1962); Sunagawa, G. et al. *Chem. Pharm. Bull.* 9, 81 (1961); Van Tamelen, E. E. et al. *Tetrahedran* 14, 8-34 (1961); Evans, D. E. et al. *JACS* 103, 5813 (1981)). Those skilled in the art will appreciate that the chemical synthesis of estradiol can be modified to include 7-membered rings by making appropriate changes to the starting materials, so that ring closure yields seven-membered rings. Estradiol or estradiol derivatives can be modified to include appropriate chemical side groups according to the invention by known chemical methods (*The Merck Index*, 11th Ed., Merck & Co., Inc., Rahway, NJ USA (1989), pp. 583-584).

Administration

The compositions described above can be provided as physiologically acceptable formulations using known techniques, and these formulations can be administered by standard routes. In general, the combinations may be administered by the topical, oral, rectal or parenteral (e.g., intravenous, subcutaneous or intramuscular) rout .

A tablet may b made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing, in a suitable machine, the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, preservative, surface-active or dispersing agent. Molded tables may be made by molding, in a suitable machine, a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally coated or scored and may be formulated so as to provide a slow or controlled release of the active ingredient therein.

Formulations suitable for topical administration in the mouth include lozenges comprising the ingredients in a flavored basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the ingredient to be administered in a suitable liquid carrier.

Formulations suitable for topical administration to the skin may be presented as ointments, creams, gels and pastes comprising the ingredient to be administered in a pharmaceutical acceptable carrier. A preferred topical delivery system is a transdermal patch containing the ingredient to be administered.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising, for example, cocoa butter or a salicylate.

Formulations suitable for nasal administration, wherein the carrier is a solid, include a coarse powder having a particle size, for example, in the range of 20 to 500 microns which is administered in the manner in which snuff is taken, i.e., by rapid inhalation through the nasal passage from a container of th powder held close up to the nose. Suitable formulations, wherein the

Example 1:

Figure 1 illustrates the inhibition of tubulin polymerization by 2-methoxyestradiol.

A. Each reaction mixture (all concentrations refer to the final reaction volume of 0.25 ml) contained 1.0 M monosodium glutamate (pH 6.6), 1.0 mg/ml (10 μM) tubulin, 1.0 mM $MGCl_2$, 4% (v/v) dimethylsulfoxide, and either 0 (curve 1), 20 μM (curve 2), 40 μM (curve 3), or 75 μM (curve 4) 2-methoxyestradiol. The 0.24 ml reaction mixtures were incubated for 15 min at 37°C and chilled on ice. After addition of 10 μl of 2.5 mM GTP the reaction mixtures were transferred to cuvettes held at 0°C, and baselines were established. At time zero the temperature controller was set at 37°C. At the times indicated by the vertical dashed lines the temperature controller was set at the indicated temperatures.

B. Each reaction mixture contained 0.8 M monosodium glutamate (pH 6.6), 1.2 mg/ml (12 μM) tubulin, 4% (v/v) dimethylsulfoxide, and either 0 (curve 1), 1.0 μM (curve 2), 2.0 μM (curve 3), 3.0 μM (curve 4), or 4.0 μM (curve 5) 2-methoxyestradiol. The 0.24 ml reaction mixtures were incubated for 15 min at 26°C and chilled on ice. After addition of 10μl of 10 mM GTP the reaction mixtures were transferred to cuvettes held at 0°C, and baselines were established. At time zero the temperature controller was set at 26°C. At the time indicated by vertical dashed line the temperature controller was set at 0°C.

Table 1

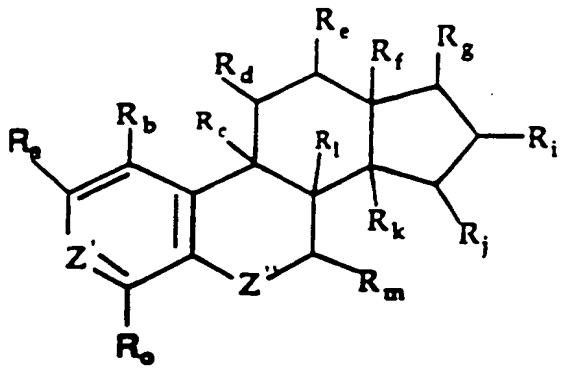
Estrogenic Compound	IC_{50} (μM ± S.D.)
2-Methoxyestradiol	1.9 ± 0.2
Diethylstilbestrol	2.4 ± 0.4
2-Bromoestradiol	4.5 ± 0.6
2-Methoxyestrone	8.8 ± 1
17-Ethynylestradiol	10.0 ± 2
2-Fluoroestradiol	27.0 ± 6
Estradiol	30.0 ± 6
Estrone	> 40
2-Methoxy-17-ethynylestradiol	> 40
Estriol	> 40
2-Methoxyestriol	> 40
Estradiol-3-O-methyl ether	> 40
2-Methoxyestradiol-3-O-methyl ether	> 40
4-Methoxyestradiol	> 40
4-Methoxyestradiol-3-O-methyl ether	> 40

Plant Products	IC_{50} (μM ± S.D.)
Colchicine	0.80 ± 0.07
Podophyllotoxin	0.46 ± 0.02
Combretastatin A-4	0.53 ± 0.05
Dihydrocombretastatin A-4	0.63 ± 0.03

IC_{50} values are defined as the concentration of an estradiol derivative required to inhibit tubulin polymerization by 50%. IC_{50} values were obtained in at least two independent experiments for non-inhibitory agents (IC_{50} > 40 μM) and at least three independent experiments for inhibitory compounds. IC_{50} values were obtained graphically, and average values are presented. S.D., standard deviation.

Claims

1. A method of making a medicament which is capable of inhibiting abnormal cell mitosis, said medicament comprising, in a pharmaceutically acceptable carrier, a cell mitosis-inhibiting compound of the formula:

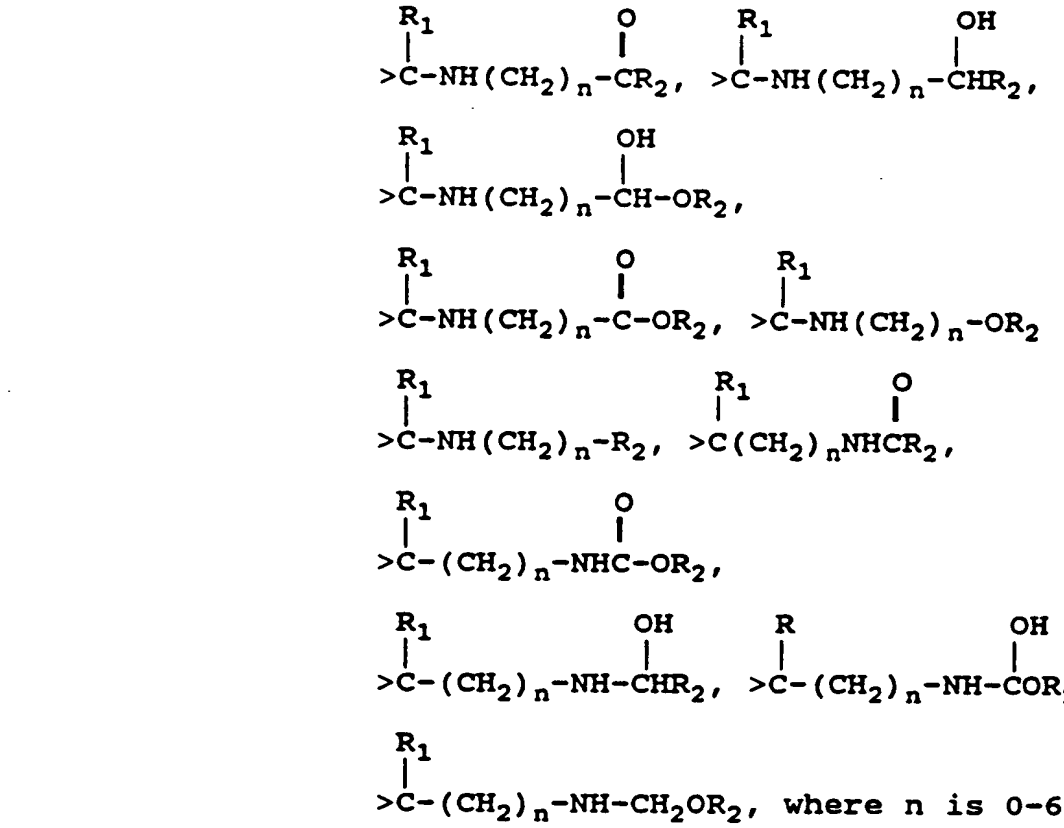


wherein:

I. R_a-R_o are defined as follows:

A) each R_a, R_b, R_c, R_d, R_e, R_f, R_l, R_j, R_k, R_l, R_m, R_o, independently is $-R_1$, $-OR_1$, $-OCOR_1$, $-SR_1$, -F, $-NHR_2$,-Br, or -I; and R_g is $-R_1$, $-OR_1$, $-OCOR_1$, $-SR_1$, -F, $-NHR_2$, -Br, -I, or -C≡CH;

or



or

B) Z" is $-Y-\underset{\underset{R_p}{|}}{CH}-$ or $-\underset{\underset{R_p}{|}}{CH}-Y-$ where R_p is $-R_1$, $-OR_1$, $-SR_1$, $-F$, $-NHR_2$, $-Br$ or $-I$;

and

IV. provided that when each R_b, R_c, R_d, R_e, R_i, R_j, R_k, R_l, R_m and R_o is H;
R_f is $-CH_3$;
R_g is $-OH$;
Z' is >COH; and
Z" is $>CH_2$;
then R_a is not $-H$;

where, in each formula set forth above, each R_1 and R_2 independently is -H, or substituted or unsubstituted alkyl, alkenyl or alkynl group of 1-6 carbons.

II. Z′ is defined as follows:

A) Z′ is X, where X is $>COR_1$, $>C(=O)-R_1$,

$>C(=O)-OR_1$, $>C(OH)-R_1$, $>C(OH)-C-OR_1$;

or

B) Z′ is $=C(R_n)-X'-$ or $-X'-C(R_n)=$, where R_n is $-R_1$, $-OR_1$, $-SR_1$, $-F$, $-NHR_2$, $-Br$ or $-I$, and X′ is X, as defined above; or X′ is also $>C=O$;

and

III. Z" is defined as follows:

A) Z" is Y, where Y is $-O-$, $-N(R_1)-$, $>CHR_1$,

$>C=O$, $>C(R_1)-(CH_2)_nOR_2$,

$>C(R_1)-(CH_2)_n-CR_2$, $>C(R_1)-(CH_2)_n-C(=O)-OR_2$,

$>C(R_1)-(CH_2)_n-CHR_2$, $>C(R_1)-(CH_2)_n-CH(OH)-OR_2$,

$>C(R_1)-NH(CH_2)_n-CR_2$, $>C(R_1)-NH(CH_2)_n-CHR_2$,

$>C(R_1)-NH(CH_2)_n-CH(OH)-OR_2$,

$>C(R_1)-NH(CH_2)_n-C(=O)-OR_2$, $>C(R_1)-NH(CH_2)_n-OR_2$,

wherein:

I. R_a-R_o are defined as follows:

A) each R_a, R_b, R_c, R_d, R_e, R_f, R_i, R_j, R_k, R_l, R_m, R_o independently is $-R_1$, $-OR_1$, $-OCOR_1$, $-SR_1$, $-F$, $-NHR_2$, $-Br$, or $-I$; and R_g is $-R_1$, $-OR_1$, $-OCOR_1$, $-SR_1$, $-F$, $-NHR_2$, $-Br$, $-I$ or $-C\equiv CH$;

or

B) each R_a, R_b, R_c, R_f, R_k, R_l, independently is $-R_1$, $-OR_1$, $-OCOR_1$, $-SR_1$, $-F$, $-NHR_2$, $-Br$, or $-I$; and each R_d, R_e, R_i, R_j, R_m, R_o independently is $=O$, $-R_1$, $-OR_1$, $-OCOR_1$, $-SR_1$, $-F$, $-NHR_2$, $-Br$, or $-I$; and R_g is $=O$, $-R_1$, $-OR_1$, $-OCOR_1$, $-SR_1$, $-F$, $-NHR_2$, $-Br$, $-I$ or $-C\equiv CH$;

and

II. Z is defined as follows:

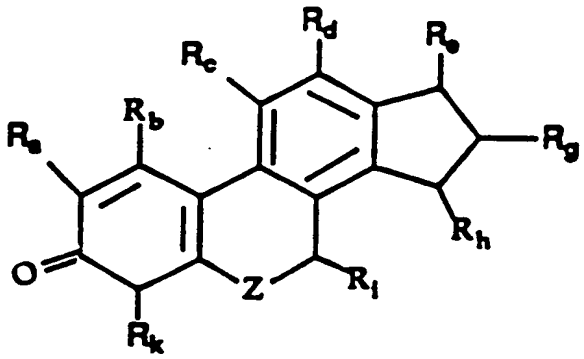
A) Z is Y, where Y is $-O-$, $-\overset{R_1}{\underset{|}{N}}-$, $>CHR_1$,

$>C=O$, $>\overset{R_1}{\underset{|}{C}}-(CH_2)_nOR_2$,

$>\overset{R_1}{\underset{|}{C}}-(CH_2)_n-\overset{O}{\overset{\|}{C}}R_2$, $>\overset{R_1}{\underset{|}{C}}-(CH_2)_n-\overset{O}{\overset{\|}{C}}-OR_2$,

$>\overset{R_1}{\underset{|}{C}}-(CH_2)_n-\overset{OH}{\underset{|}{C}}HR_2$,

$>\overset{R_1}{\underset{|}{C}}-(CH_2)_n-\overset{OH}{\underset{|}{C}}H-OR_2$,



wherein:

I. R_a-R_k are defined as follows:

A) each R_a, R_b, R_c, R_d, R_g, R_h, R_i, R_k independently is $-R_1$, $-OR_1$, $-OCOR_1$, $-SR_1$, $-F$, $-NHR_1$, $-Br$, or $-I$; and R_e is $-R_1$, $-OR_1$, $-OCOR_1$, $-SR_1$, $-F$, $-NHR_1$, $-Br$, $-I$ or $-C\equiv CH$;

or

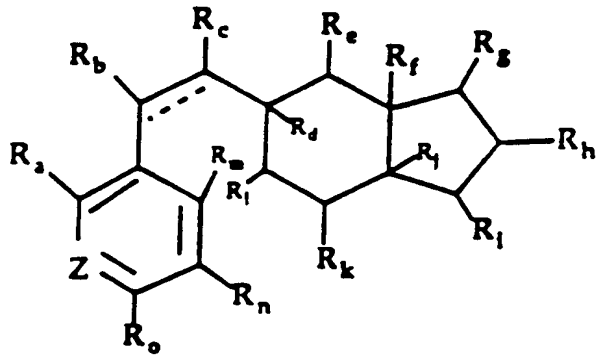
B) each R_a, R_b, R_c, R_d, independently is $-R_1$, $-OR_1$, $-OCOR_1$, $-SR_1$, $-F$, $-NHR_1$, $-Br$, or $-I$ and each R_g, R_h, R_i, R_k independently is $=O$, $-R_1$, $-OR_1$, $-OCOR_1$, $-SR_1$, $-F$, $-NHR_1$, $-Br$ or $-I$; and R_e is $=O$, $-R_1$, $-OR_1$, $-OCOR_1$, $-SR_1$, $-F$, $-NHR_1$, $-Br$, $-I$ or $-C\equiv CH$;

and

II. Z is defined as follows:

where, in each formula set forth above, each R_1 and R_2 independently is -H, or substituted or unsubstituted alkyl, alkenyl or alkynl group of 1-6 carbons.

5. A method of making a medicament which is capable of inhibiting abnormal cell mitosis, said medicament comprising, in a pharmaceutically acceptable carrier, a cell mitosis-inhibiting compound of the formula:



wherein:

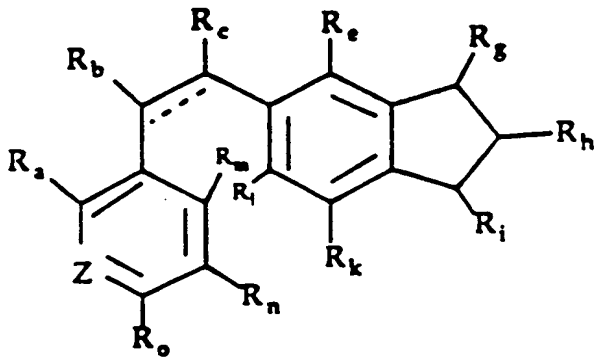
I. R_a-R_o are defined as follows:

A) each R_a, R_b, R_c, R_d, R_e, R_f, R_g, R_h, R_j, R_k, R_l, R_m, R_n, R_o independently is $-R_1$, $-OR_1$, $-OCOR_1$, $-SR_1$, -F, $-NHR_2$, -Br, or -I; and R_i is $-R_1$, $-OR_1$, $-OCOR_1$, $-SR_1$, -F, $-NHR_2$, -Br, -I or -C≡CH;

or

B) each R_a, R_d, R_f, R_j, R_m, R_n, R_o independently is $-R_1$, $-OR_1$, $-OCR_1$, $-SR_1$,

6. A method of making a medicament which is capabl of inhibiting abnormal cell mitosis, said medicament comprising, in a pharmaceutically acceptable carrier, a cell mitosis-inhibiting compound of the formula:



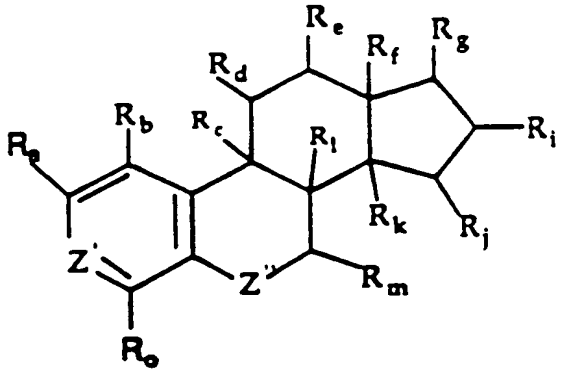
wherein:

I. R_a-R_o are defined as follows:

A) each R_a, R_b, R_c, R_e, R_g, R_h, R_k, R_l, R_m, R_n, R_o independently is $-R_1$, $-OR_1$, $-OCOR_1$, $-SR_1$, -F, $-NHR_2$, -Br, or -I; and R_i is $-R_1$, $-OR_1$, $-OCOR_1$, $-SR_1$, -F, $-NHR_2$, -Br, -I or -C≡CH;

or

B) each R_a, R_e, R_l, R_m, R_n, R_o independently is $-R_1$, $-OR_1$, $-OCOR_1$, $-SR_1$, -F, $-NHR_2$, -Br, -I and each R_b, R_c, R_g, R_h is =O,



wherein:

I. R_a-R_o are defined as follows:

(A) each R_a, R_b, R_c, R_d, R_e, R_f, R_i, R_j, R_k, R_l, R_m, R_o, independently is $-R_1$, $-OR_1$, $-OCOR_1$, $-SR_1$, -F, $-NHR_2$,-Br, or -I; and R_g is $-R_1$, $-OR_1$, $-OCOR_1$, $-SR_1$, -F, $-NHR_2$, -Br, -I or -C≡CH;

or

(B) each R_a, R_b, R_c, R_f, R_k, R_l, R_o, is $-R_1$, $-OR_1$, $-OCOR_1$ $-SR_1$, -F, $-NHR_2$, -Br, or -I; and each R_d, R_e, R_i, R_j, R_m, independently is =O, $-R_1$, $-OR_1$, $-OCOR_1$, $-SR_1$, -F,$-NHR_2$, -Br or -I; and R_g is =O, $-R_1$, $-OR_1$, $-OCOR_1$, $-SR_1$, -F, $-NHR_2$, -Br, -I or -C≡CH;

$>C(R_1)-NH(CH_2)_n-R_2$, $>C(R_1)(CH_2)_nNHCR_2$,

$>C(R_1)-(CH_2)_n-NHC(=O)-OR_2$,

$>C(R_1)-(CH_2)_n-NH-CH(OH)R_2$, $>C(R)-(CH_2)_n-NH-C(OH)R_2$, or

$>C(R_1)-(CH_2)_n-NH-CH_2OR_2$, where n is 0-6;

or

B) Z" is $-Y-CH(R_p)-$ or $-CH(R_p)-Y-$ where R_p is $-R_1$, $-OR_1$, $-SR_1$, $-F$, $-NHR_2$, $-Br$ or $-I$;

provided that when:

3) each R_b, R_c, R_d, R_e, R_j R_k, R_l, R_m, is $-H$;

R_f is $-CH_3$;

R_g is $-OH$, $-OCCH_3$;

R_i is $-H$, $-OH$, or $=O$;

R_o is $-H$ or $-Br$;

Z' is $>COH$; and

Z" is $>CH_2$ or $-OH$; then

R_a is not $-F$, $-Br$, $-OH$ or $-H$;

and

4) each R_b, R_c, R_d, R_e, R_i, R_j R_k, R_l, R_m, is $-H$;

R_f is $-CH_3$;

R_g is $-OH$; and

Z" is $>CH_2$; then

wherein:

I. R_a-R_k are defined as follows:

A) each R_a, R_b, R_c, R_d, R_g, R_h, R_i, R_k independently is $-R_1$, $-OR_1$, $-OCOR_1$, $-SR_1$, -F, $-NHR_2$, -Br, or -I; and R_e is $-R_1$, $-OR_1$, $-OCOR_1$, $-SR_1$, -F, $-NHR_2$, -Br, -I or -C≡CH;

or

B) each R_a, R_b, R_c, R_d, R_k, is $-R_1$, $-OR_1$, $-OCOR_1$, $-SR_1$, -F, $-NHR_2$, -Br, or -I; and each R_g, R_h, R_i, independently is =O, $-R_1$, $-OR_1$, $-OCOR_1$, $-SR_1$, -F, -Br, or -I; and R_e is =O, $-R_1$, $-OR_1$, $-OCOR_1$, $-SR_1$, -F, -Br, -I or -C≡CH;

and

I. Z′ is defined as follows:

A) Z′ is X, where X is $>COR_1$, $>C_2C(=O)-R_1$,

$>C_2C(=O)-OR_1$, $>CC(OH)-R_1$, $>C(OH)-C(OH)-OR_1$;

or

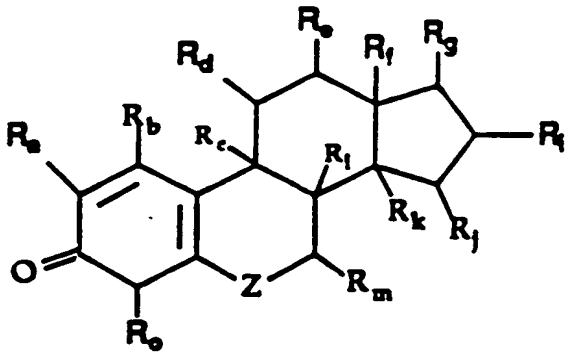
B) Z′ is $=C(R_n)-X'-$ or $-X'-C(R_n)=$, where R_n is $-R_1$, $-OR_1$, $-SR_1$, -F, $-NHR_2$, -Br or -I, and X′ is X, as defined above; or X′ is also >C=O;

and

II. Z" is defined as follows:

where, in each formula set forth above, each R_1 and R_2 independently is -H, or substituted or unsubstituted alkyl, alkenyl or alkynl group of 1-6 carbons.

9. A compound of the general formula below, said compound being a cell-mitosis-inhibiting compound:



wherein:

I. R_a-R_o are defined as follows:

A) each R_a, R_b, R_c, R_d, R_e, R_f, R_i, R_j, R_k, R_l, R_m, R_o independently is $-R_1$, $-OR_1$, $-OCOR_1$, $-SR_1$, -F, $-NHR_2$, -Br, or -I; and R_g is $-R_1$, $-OR_1$, $-OCOR_1$, $-SR_1$, -F, $-NHR_2$, -Br, -I or -C≡CH;

or

B) each R_a, R_b, R_c, R_f, R_k, R_l, independently is $-R_1$, $-OR_1$, $-OCOR_1$, $-SR_1$, -F, $-NHR_2$, -Br, or -I; and each R_d, R_e, R_i, R_j, R_m, R independently is =O, $-R_1$, $-OR_1$, $-OCOR_1$,

$>C(R_1)-(CH_2)_n-NH-CH(OH)R_2$, $>C(R)-(CH_2)_n-NH-C(OH)R_2$, or

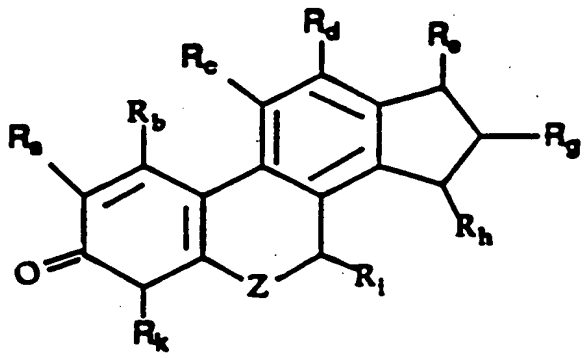
$>C(R_1)-(CH_2)_n-NH-CH_2OR_2$, where n is 0-6;

or

B) Z is $-Y-CH(R_n)-$ or $-CH(R_n)-Y-$, where R_n is $-R_1$, $-OR_1$, $-SR_1$, $-F$, $-NHR_2$, $-Br$ or $-I$;

where, in each formula set forth above, each R_1 and R_2 independently is -H, or substituted or unsubstituted alkyl, alkenyl or alkynl group of 1-6 carbons.

10. A compound of the general formula below, said compound being a cell-mitosis-inhibiting compound:



wherein:

I. R_a-R_k are defined as follows:

A) each R_a, R_b, R_c, R_d, R_g, R_h, R_i, R_k independently is $-R_1$, $-OR_1$, $-OCOR_1$, $-SR_1$,

$>C-(CH_2)_n-NH-CHR_2$, $>C-(CH_2)_n-NH-COR_2$, or

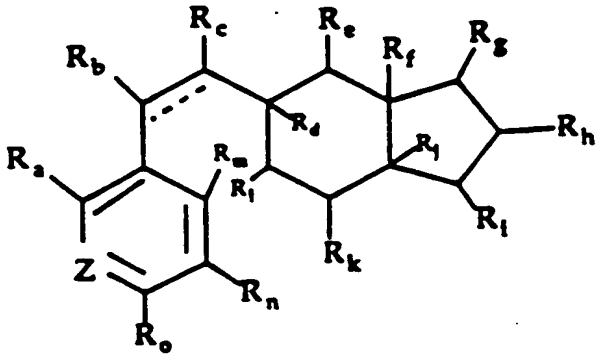
$>C-(CH_2)_n-NH-CH_2OR_2$, where n is 0-6;

or

Z is $-Y-CH-$ or $-CH-Y-$, where R_n is $-R_1$, $-OR_1$, $-SR_1$, $-F$, $-NHR_2$, $-Br$ or $-I$;

where, in each formula set forth above, each R_1 and R_2 independently is -H, or substituted or unsubstituted alkyl, alkenyl or alkynl group of 1-6 carbons.

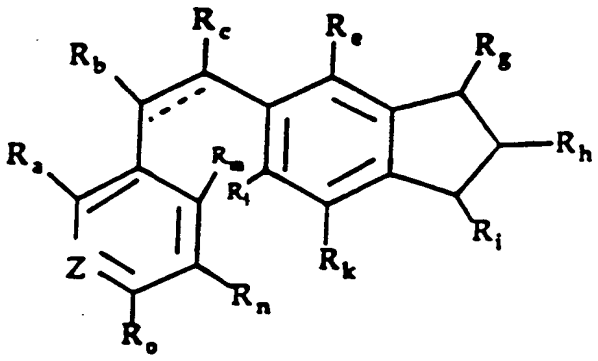
11. A compound of the general formula below, said compound being a cell-mitosis-inhibiting compound:



Z is $=C(R_p)-X'-$ or $-X'-C(R_p)=$, where R_p is $-R_1$, $-OR_1$, $-SR_1$, $-F$, $-NHR_2$, $-Br$ or $-I$; and X' is X, as defined above; or X' is $>C=O$;

where, in each formula set forth above, each R_1 and R_2 independently is -H, or substituted or unsubstituted alkyl, alkenyl or alkynl group of 1-6 carbons; and the bond indicated by C•••C is absent or, in combination with the C—C bond is the unit HC=CH.

12. A compound of the general formula below, said compound being a cell-mitosis-inhibiting compound:



wherein:

I. R_a-R_o are defined as follows:

where, in each formula set forth above, each R_1 and R_2 ind pendently is -H, or substituted or unsubstituted alkyl, alkenyl or alkynl group of 1-6 carbons; and the bond indicated by C•••C is absent or, in combination with the C—C bond is the unit HC=CH.

13. The method of claim 1, wherein said cell-mitosis-inhibiting compound is 2-methoxyestradiol.

14. The method of claim 1, wherein said cell-mitosis-inhibiting compound is 2-fluoroestradiol.

15. The method of claim 1, wherein said cell-mitosis-inhibiting compound is 2-bromoestradiol.

16. The method of claim 1, wherein said cell-mitosis-inhibiting compound is 2-methoxyestrone.

17. The method of claim 1, wherein said cell-mitosis-inhibiting compound is 17-ethynylestradiol.

18. The method of claims 1 or 2 wherein said compound is further characterized in that

A) Z′ is $=C(R_n)-X'-$ or $-X'-C(R_n)=$; and
Z" is $-Y-CH(R_p)-$ or $-CH(R_p)-Y-$; or

B) Z′ is X; and Z" is $-Y-CH(R_p)-$ or $-CH(R_p)-Y-$; or

C) Z′ is $=C(R_n)-X'-$ or $-X'-C(R_n)=$; and Z" is Y.

25. The compound of any one of claims 7-12, wherein at least one of R_a-R_p is $-OCH_3$.

INTERNATIONAL SEARCH REPORT

Internati nal application No.
PCT/US94/08767

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 31/56; C07J 41/00, 31/00, 13/00, 9/00, 5/00, 7/00, 3/00, 1/00.

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

514/177, 178, 179, 182; 552/516, 522, 523, 524, 525, 535, 536, 540, 541, 542, 543, 544, 548, 549, 550, 551, 552, 553, 554, 555, 557, 558, 559, 560, 562. 563, 564, 565, 566, 567, 569, 571, 572, 573, 575, 582, 583, 584, 585, 599, 603, 604, 605, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 623, 624, 625, 626, 627, 628, 629, 642, 643, 644, 646, 647, 650, 651, 652.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

Group I, claims 1, 3, 7, 9, and 24, each in part, and claims 13-17; directed to a method of making a medicament compound in which the A ring is aromatic and said compound.

Group II, claims 1, 7, 9, 18, 21, each in part, and 25, directed to a method of making a medicament in which the A ring is aromatic and Z" is Y and Y is O.

Group III, claims 1, 7, 9, 18, 21, each in part, and 25, as in Group II, except that Y is N.

Group IV, claims 1, 3, 7, 18, 21, each in part, and 25, as in Group II, except that the A ring is aromatic and contains 7 carbons and the B ring contains 6 carbon atoms.

Group V, claims 1, 3, 7, 9, 18, 21, each in part, and 25, as in Group IV except that the B ring contains 7 carbon atoms.

Group VI, claims 1 and 18, each in part, in which the A ring contains 6 carbon atoms and the B ring contains 7 carbon atoms.

Group VII, claims 1, 3, 7, 18, 25, each in part, and 25, in which A ring is 7 carbon aromatic and the B ring contains carbons, Z" is Y and Y is O.

Group VIII, claims 1, 3, 7, 18, 21, each in part, and 25, as in
Group VII in which Y is N.

Group IX, claims 2, 4, 8, 10, 18, 21, each in part, and 25, directed to a method of making a medicament in which the A and C rings are each aromatic.

Group X, claims 2, 8, 10, 18, 21, each in part, and 25, directed to a method of making a medicament compound as in Group IX in which the Z" is Y and Y is O.

Group XI, claims 2, 8, 10, 18, 21, each in part, and 25, as in Group IX in which Y is N.

Group XII, claims 2, 8, 18, 21, each in part, and 25, as in Group IX in which the A ring contains 7 carbon atoms.

Group XIII, claims 2 and 18, each in part, as in Group VIII, in which the B ring contains 7 carbon atoms.

Group XIV, claims 2, 18, 25, each in part, and 25 as in Group XII, in which the B ring contains 7 atoms.

Group XV, claims 2, 8, 10, 18, each in part, and 25 in which the B ring has 7 carbons and Y is O.

Group XVI, claims 2, 8, 18, each in part, and 25 in which the B ring has 7 carbons and Y is N.

Group XVII, claims 3 in part, 19, and 24, directed to a method of making a medicament in which there is a keto group at C3, the A ring is aromatic and the B ring contains 6 carbon atoms.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/08767

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classificati n (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/177, 178, 179, 182; 552/558, 614, 617, 625, 627

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS online

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	J. Steroid Biochem., Vol. 32, No. 6, issued 1989, J. Seegers et al., "The Cytotoxic Effects of Estradiol-17beta, catecholestradiols and methoxyestradiols on dividing MCF-7 and HeLa cells" pages 797-809, see entire article.	1, 7, 13 ---------- 14, 15, 16, 17, 24
X -- Y	Chemical Abstracts, Vol. 105, issued 1986, W.J. Wheeler et al., "Mitotic inhibition and aneuploidy induction by naturally occurring and synthetic estrogens in Chinese hamster cells in vitro", see abstract no. 54822, Mutat. Res., 171(1), 1986, 31-41.	1, 17 -------- 13, 14, 15, 16, 24

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
20 OCTOBER 1994

Date of mailing of the international search report
10 NOV 1994

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Form PCT/ISA/210 (second sheet)(July 1992)*

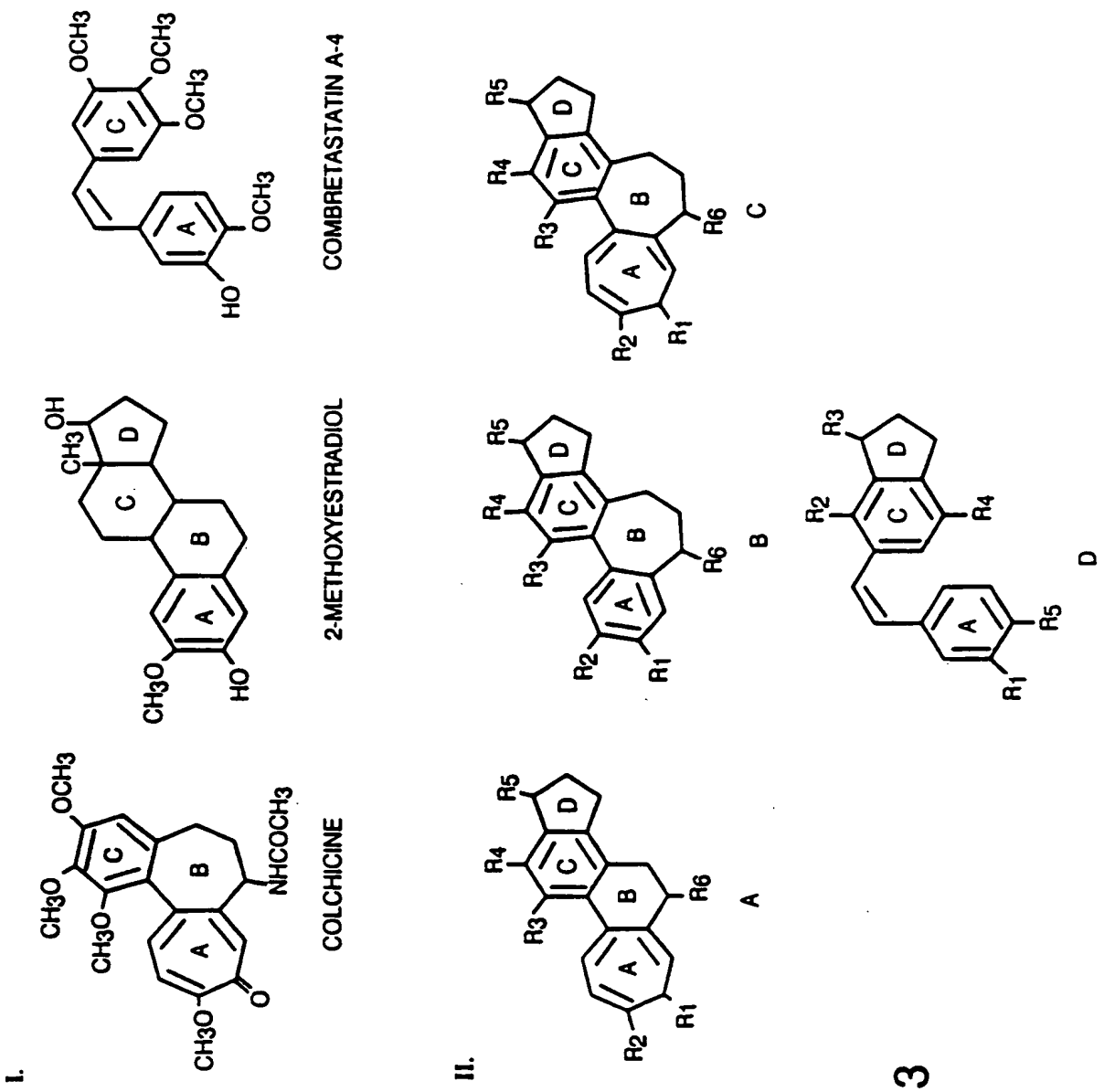
I.
COLCHICINE
2-METHOXYESTRADIOL
COMBRETASTATIN A-4
II.
A
B
C
D

FIG. 3

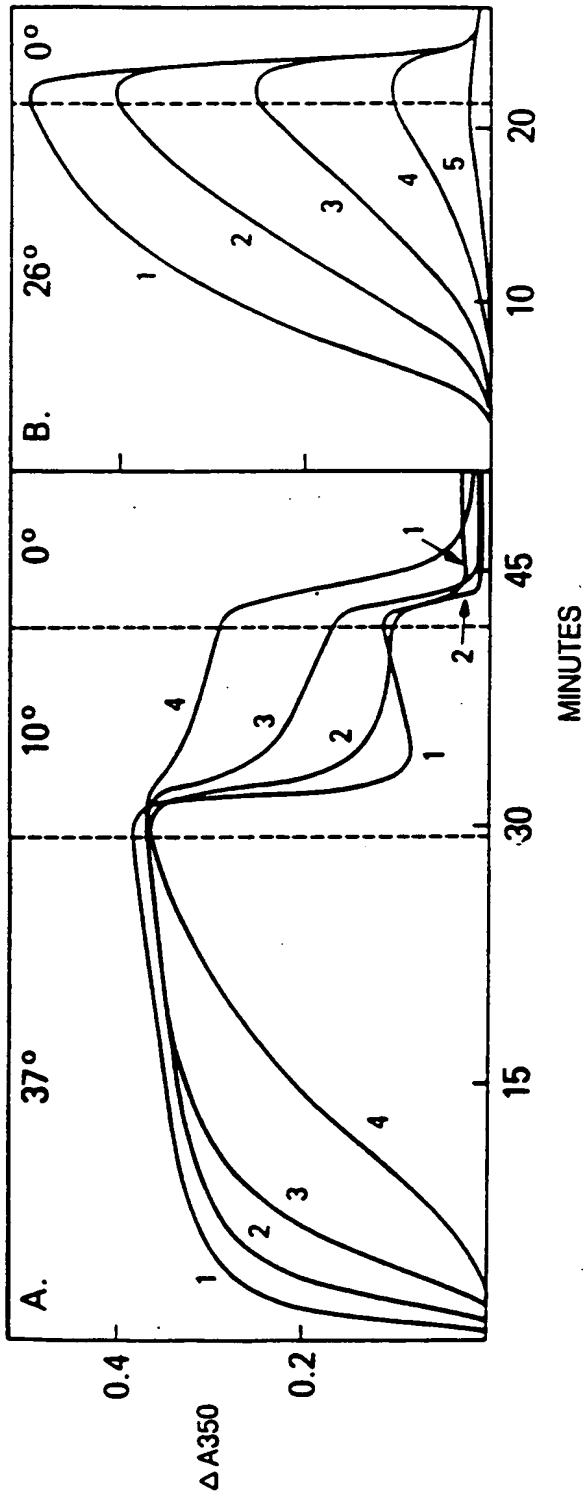

FIG. 1

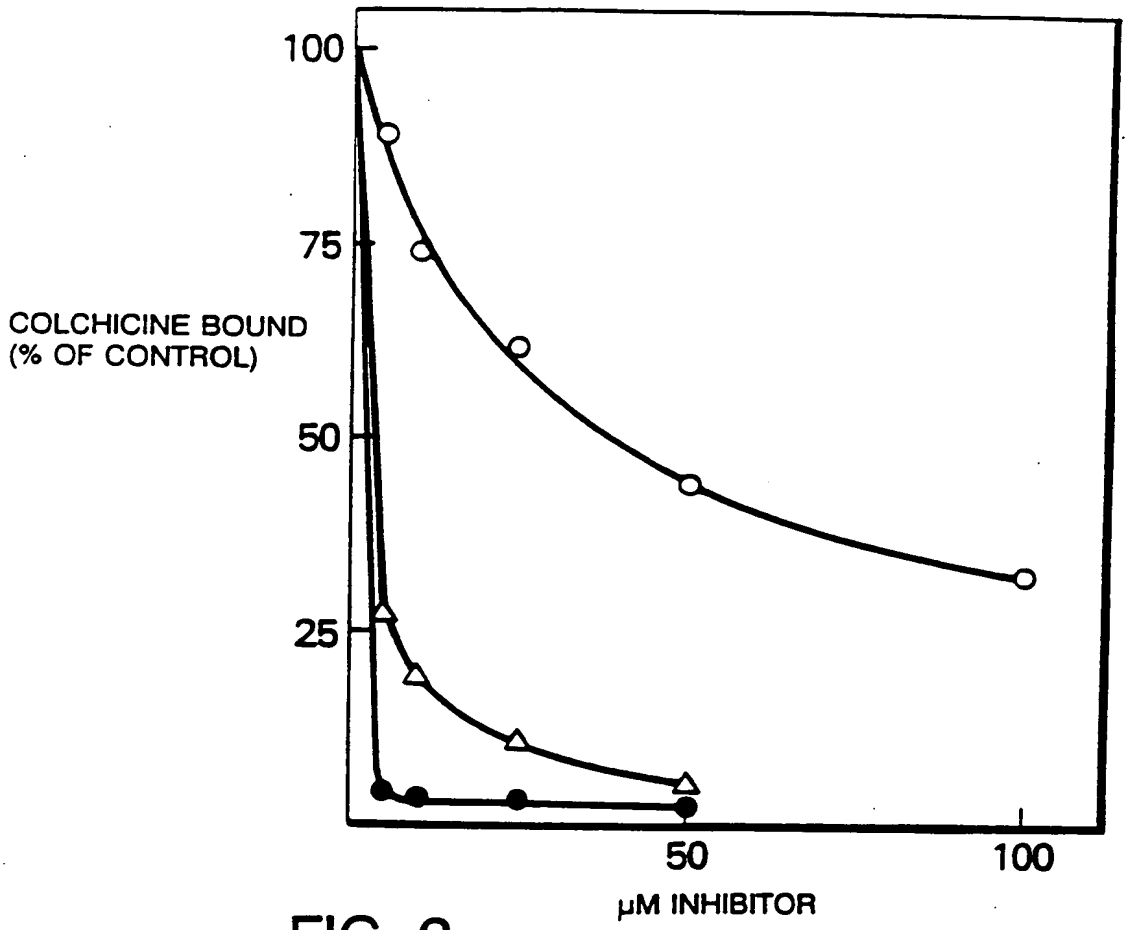

FIG. 2